The multiplicity of combinations of myosin light chains and heavy chains in histochemically typed single fibres

Rabbit tibialis anterior muscle

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1. Combined histochemical and biochemical single-fibre analyses [Staron & Pette (1987) *Biochem. J.* **243**, 687–693], were used to investigate the rabbit tibialis-anterior fibre population. 2. This muscle is composed of four histochemically defined fibre types (I, IIC, IIA and IIB). 3. Type I fibres contain slow myosin light chains LC1s and LC2 and the slow myosin heavy chain HCl, and types IIA and IIB contain the fast myosin light chains LC1f, LC2f and LC3f and the fast heavy chains HCIIa and HCIIb respectively. 4. A small fraction of fibres (IIAB), histochemically intermediate between types IIA and IIB, contain the fast light myosin chains but display a coexistence of HCIIa and HCIIb. 5. Similarly to the soleus muscle, C fibres in the tibialis anterior muscle contain both fast and slow myosin light chains and heavy chains. The IIC fibres show a predominance of the fast forms and the IC fibres (histochemically intermediate between types I and IIC) a predominance of the slow forms. 6. A total of 60 theoretical isomyosins can be derived from these findings on the distribution of fast and slow myosin light and heavy chains in the fibres of rabbit tibialis anterior muscle.

INTRODUCTION

In the preceding study (Staron & Pette, 1987) we presented evidence for a pronounced myosin polymorphism in four histochemically defined fibre types (I, IC, IIC and IIA) of adult rabbit soleus muscle. To extend the biochemical characterization of known fibre types, the present study investigates the fibre populations of adult rabbit tibialis anterior muscle. This muscle contains the histochemical (myosin ATPase-based) fibre types I, IIC, IIA and IIB. In addition to studying the IIB fibre population, it was of interest to compare the fibre types common to both the soleus and the tibialis anterior muscles. Also, in view of histochemical (Andersen & Henriksson, 1977; Jansson *et al.*, 1978; Ingjer, 1979; Staron *et al.*, 1983; White & Snow, 1985) and immunohistochemical (Gauthier & Lowey, 1979; Lutz *et al.*, 1979; Billeter *et al.*, 1980; Pierobon-Bormioli *et al.*, 1981) evidence for a population between types IIA and IIB (IIAB), we considered it worthwhile to apply our technique of combined histochemical and biochemical analyses to investigate these fibres.

MATERIALS AND METHODS

Three normal tibialis anterior muscles were taken from adult male New Zealand White rabbits. The muscles were cut into strips of approx. 3 mm thickness and were quickly frozen in a slightly stretched position in melting isopentane (–159 °C).

The processing of the tissue for myosin ATPase histochemistry on thin cross-sections and isolation of defined fibre pieces from freeze-dried thick serial cross-sections were essentially the same as described in

the preceding study (Staron & Pette, 1987). However, because of the tibialis-anterior fibre architecture, six thin (12 µm) serial cross-sections were alternated with one thick (200–300 µm) cross-section until 12–16 thick sections were obtained. Over the total distance covered (4–6 mm), some fibres did not run the full length. It was therefore important to verify the fibre population and ATPase activity continuously by using frequent thin sections.

Microelectrophoresis and electrophoresis of the heavy chains (HC) were performed as previously described (Staron & Pette, 1987). Two-dimensional electrophoresis of myofibrillar proteins was performed in accordance with O'Farrell (1975). Peptide mapping of the myosin heavy chains was performed by using the method of Cleveland *et al.* (1977) after limited proteolysis by *Staphylococcus aureus* V8 proteinase (Miles Laboratories, Elkhart, IN, U.S.A.). All gels were stained by using the method of Oakley *et al.* (1980). Approximate molecular masses and identification of peptide bands were determined by using marker proteins and purified myosin prepared from rabbit soleus and psoas muscles.

RESULTS

The rabbit tibialis anterior muscle is composed of four histochemically defined fibre types, types I, IIC, IIA and IIB (Fig. 1). Of these, types I and IIC make up minor populations. In addition, a few fibres were observed that stained intermediately between types I and IIC (IC) and also between types IIA and IIB (IIAB) (Fig. 1b).

The myosin profile of type I fibres consisted of the slow HCl (Fig. 2a) and slow LC1s and LC2s. All type I fibres

Abbreviations used: HC, heavy chain; LC, light chain.

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Fig. 1. Myosin-ATPase-based fibre types in rabbit tibialis anterior muscle

Five fibre types (types I, IIC, IIA, IIAB and IIB) are distinguished in serial cross-sections stained for myosin ATPase after preincubations at various pH values. Key: I, type I; C, type IIC; A, type IIA; AB, type IIAB; B, type IIB. Magnification $\times 160$.

appeared the same and displayed both LC1s_a and LC1s_b, the latter being more prominent (Fig. 2b). The IIA and IIB fibres were identical with regard to their LC composition (Fig. 2b). Likewise, two-dimensional electrophoreses (not shown) confirmed the presence of LC1f, LC2f and LC3f and the absence of LC1s_a and LC1s_b in the IIA and IIB fibres. Therefore a band observed in the region of LC1s_a (Fig. 2b) was not the slow light chain in these fast fibres.

The IIA and IIB fibres contained different HC forms. With the use of a low-percentage polyacrylamide gel, reproducible minute differences in the migration rates of the HC forms were observed (Figs. 2a and 3). The HCIIb was slightly faster than the HCIIa (Fig. 3). When co-electrophoresed, these two HC forms appear as one larger band (results not shown). This difference between HCIIa and HCIIb was further substantiated by peptide mapping patterns (Fig. 4).

The IIAB fibres, histochemically intermediate between types IIA and IIB, also contained the set of fast myosin LC forms (Fig. 2b). The migration velocity of the HC of these fibres was variable and could not always be classified as either the HCIIa or the HCIIb. However, peptide mapping performed on single and pooled IIAB fibres revealed a mixed HCIIa/HCIIb composition (Fig. 4).

Because of the small percentage of C fibres in normal tibialis anterior muscle, we were unable to investigate them in the large numbers. However, the C fibres analysed contained both fast and slow myosins (LC and HC forms) in various proportions. These fibres were similar to the C fibres observed in the soleus muscle (Staron & Pette, 1986, 1987), with some resembling type I (IC) and others IIA fibres (IIC). As judged from the

migration rate, the slower-migrating band in the C fibres apparently corresponded to HCIIa and the faster-migrating band to HCI (Fig. 2a).

The 29–30 kDa peptide, which was present in all soleus fibres (Staron & Pette, 1987), was also found in tibialis-anterior fibres. This peptide, designated X-protein, appeared to correlate with the oxidative capacity of the fibre. Therefore it was prominent in the I, IC and IIC fibres, present in variable amounts in the IIA and IIAB fibres and not detectable in the IIB fibres (Fig. 2b).

DISCUSSION

In the normal rabbit tibialis anterior muscle four fibre types can be distinguished histochemically: types I, IIC, IIA and IIB. In addition, there exist fibres intermediate between types I and IIC (IC fibres) and between types IIA and IIB (IIAB fibres). These findings support the suggestion that transitions occur between types I and IIB (Jansson *et al.*, 1978; Billetter *et al.*, 1981) and that skeletal-muscle fibres are dynamic structures (Guth & Yellin, 1971) displaying a continuum between two extremes (Staron *et al.*, 1983; Pette, 1984, 1985; Pette & Spamer, 1986).

This histochemical continuum is supported by the pattern of myosin LC and HC distributions (Table 1). The present study of IIA, IIAB and IIB fibres extends the spectrum to its apparent full range in rabbit slow-twitch and fast-twitch skeletal muscle. The correlation between histochemical myosin ATPase activity and myosin HC composition in the soleus muscle (Staron & Pette, 1986, 1987) also applies to the tibialis anterior muscle in the present study. This is valid not only for the C-fibre

Fig. 2. Myosin heavy and light chains in histochemically typed single fibres of rabbit tibialis anterior muscle

Segments of the same fibre were isolated separately and analysed for their myosin heavy-chain composition (a) and for their myofibrillar peptide composition (b). Key: I, type I; C, type IIC; A, type IIA; AB, type IIAB; B, type IIB. Unlabelled lanes represent separations of myosin extracted from rabbit soleus (left) and psoas (right). Abbreviations: AC, actin; HC, myosin heavy chain; TM, tropomyosin; X, X-protein, 1f, 2f and 3f, fast myosin light chains; 1s, 2s, slow myosin light chains.

populations but also for the IIAB fibres. This correlation is further substantiated by comparing IIA fibres from soleus and tibialis anterior muscles. Both populations are histochemically identical (acid-labile, alkali-stable) and appear to both contain the HCIIa but vary with regard to their LC composition. Type IIA fibres in tibialis

Fig. 3. Myosin heavy-chain analysis of fast fibre subtypes from rabbit tibialis anterior muscle

Myosin heavy chains from histochemically typed single fibres were electrophoretically analysed under denaturing conditions in a SDS/5%-polyacrylamide gel. Types IIA (A) and IIB (B) were loaded alternately. Note the consistent migration differences.

Fig. 4. Peptide cleavage patterns of isolated myosin heavy chains from single fast fibre subtypes of rabbit tibialis anterior muscle

Segments of histochemically typed single IIA (A), IIAB (AB) and IIB (B) fibres were isolated and extracted, and their heavy chains were separated electrophoretically on a SDS/5%-polyacrylamide gel. The bands corresponding to the heavy chains were cut out and subjected to proteolytic digestion, with subsequent electrophoresis (Cleveland *et al.*, 1977). Arrows indicate peptides within the heavy chains of type IIAB common to either type IIA (→) or type IIB (←).

Table 1. Myosin light and heavy chains in histochemically typed fibres from rabbit tibialis anterior muscle

The distribution of myosin light chains (LC) and heavy chains (HC) was determined electrophoretically in fibre pieces isolated from thick serial cross-sections, according to myosin-ATPase-stained thin serial cross-sections.

Fibre type	Light chains					Heavy chains		
	LC1s	LC2s	LC1f	LC2f	LC3f	HCI	HCIIa	HCIIb
I	+	+				+		
IC	+	+	+	+	(+)	+	+	
IIC	+	+	+	+	+	+	+	
IIA			+	+	+		+	
IIAB			+	+	+		+	+
IIB			+	+	+			+

Table 2. Theoretical combinations of myosin light chains (LC) and heavy chains (HC) according to their presence in histochemically defined fibre types IIA, IIAB and IIB in rabbit tibialis anterior muscle

Type IIA contains combinations 1A–3A. Type IIAB contains combinations 1A, B, C–3A, B, C. Type IIB contains combinations 1C–3C.

Light chains		Heavy chains		
		A	B	C
1	(LC1f) ₂ (LC2f) ₂	(HCIIa) ₂	(HCIIa)(HCIIb)	(HCIIb) ₂
2	(LC1f)(LC3f)(LC2f) ₂	(HCIIa) ₂	(HCIIa)(HCIIb)	(HCIIb) ₂
3	(LC3f) ₂ (LC2f) ₂	(HCIIa) ₂	(HCIIa)(HCIIb)	(HCIIb) ₂

anterior muscle contain only fast light chains, whereas soleus-muscle IIA fibres contain the fast light chains and various sets of slow light chains (Staron & Pette, 1987). Subtle histochemical differences do appear to exist between IIA fibres in rabbit tibialis anterior and soleus muscles when comparing their staining intensities following alkali (pH 9.6) preincubation (present study and Staron & Pette, 1987) and may reflect possible HC differences (R. S. Staron & D. Pette, unpublished work).

The presence of HCIIa and HCIIb within the IIAB fibres may either reflect the presence of two HC homodimers (HCIIa)₂ and (HCIIb)₂, a HC heterodimer (HCIIa)(HCIIb) or both. Assuming the existence of the HC heterodimer, a total of nine possible fast isomyosins may exist in the IIAB fibres (Table 2). Three of these, resulting from the fast light chains and the HCIIa, are present in the IIA fibres. Likewise, three are present in the IIB fibres resulting from the fast LC forms and the HCIIb. From these nine fast isomyosin combinations, six may therefore be added to the 54 possible isomyosins derived for rabbit soleus-muscle fibres (Staron & Pette, 1987). This yields a total of 60 possible isomyosins in rabbit skeletal-muscle fibres.

The idea of so many isomyosins is highly speculative, especially because it is not clear whether or not the suggested HC heterodimers (HCI)(HCIIa) and (HCIIa)(HCIIb) exist. Only a limited number (three fast and two slow) of isomyosins have been detected by electrophoresis under non-denaturing conditions in adult fast-twitch and slow-twitch rabbit muscles (Hoh & Yeoh, 1979; d'Albis *et al.*, 1979; Pinter *et al.*, 1981). d'Albis *et al.* (1986) have demonstrated additional fast isomyosins in adult mouse

muscle. However, these findings do not approach the calculated number of possible isomyosins. This may be due to insufficient separation by available methods or predominance of particular isomyosins. Both possibilities are supported by the fuzzy appearance of the isomyosin bands in electrophoretic separations under non-denaturing conditions.

The distributional pattern of various isomyosins within the same fibre is unknown. Several locations are possible: (1) a regional random distribution with A-bands made up of thick filaments containing more than one isomyosin or by different thick filaments each composed of only one isomyosin, (2) a sarcomeric distribution with homogeneous thick filaments but heterogeneity along the myofibril, and (3) a myofibrillar distribution with isomyosin homogeneity throughout the length of the fibre. The possibility that thick filaments may be made up of different myosins has been reported for nematode muscle (Miller *et al.*, 1983). On the other hand, segmental differences in contractile properties (Edman *et al.*, 1985) and myofibrillar ATPase activity (te Kronnie *et al.*, 1985) along single frog muscle fibres have also been described. Such a distribution cannot be excluded, since our methodical approach can monitor pronounced changes in isomyosin composition along the fibres, but not subtle regional changes, e.g. from sarcomere to sarcomere.

An interesting but unresolved observation relates to the presence of an unidentified 29–30 kDa peptide in 'oxidative' fibres. According to its molecular mass (Pullan & Noltmann, 1985) and its fibre specificity (Riley *et al.*, 1982; Väänänen *et al.*, 1982; Shima *et al.*, 1983),

X-protein might represent carbonic anhydrase III. However, this enzyme is easily extracted (Pullan & Noltmann, 1985), and therefore it should be eluted by the pre-extraction procedure. Another possibility is that it represents an insoluble membrane protein, e.g. the mitochondrial ADP-ATP translocator (Klingenberg *et al.*, 1977).

In conclusion, analysis of the tibialis-anterior fibre population has demonstrated a I, IC, IIC, IIA, IIAB and IIB fibre type continuum. These fibres are characterized by various combinations of slow and fast myosins. Without consideration of the LC1s_a/LC1s_b differences, a total of 60 theoretical isomyosins may exist in the fibre population of rabbit tibialis anterior muscle. Therefore, although a particular fibre type may predominate, this spectrum reflects a broad range of adaptability.

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